Short Communication

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Antioxidant defense of zinc acetate supplementation on the brain of protein–undernourished rats

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ABSTRACT

The effect of zinc on brain weight, glutathione (GSH) level, catalase activity and lipid peroxidation in the brain of protein-undernourished (PU) rats was investigated following the ad *libitum* ingestion of zinc acetate (30 mg/L) in drinking water for two weeks. Results show that protein-undernutrition induced significant reductions ($P<0.01$) in brain weight and catalase activity while it induced significant increase ($P<0.05$) in lipid peroxidation when compared with well-nourished rats; but no significant effect was observed on GSH level. The ingestion of zinc acetate, however, by PU rats had no significant effect on brain weight but induced significant increase in catalase activity. There were also reductions ($P<0.05$) in lipid peroxidation and GSH level. These results show that zinc acts as an antioxidant but is ineffective in ameliorating some of the biochemical changes associated with protein-undernutrition-induced brain damage particularly reduction in brain weight.

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Keywords: Protein-undernutrition, Oxidative stress, Brain weight, Lipid peroxidation, Glutathione, Catalase.

INTRODUCTION

Oxidative stress is known to be an important contributing factor in many chronic diseases. Oxidative mechanisms of damage have been associated with the damage to brain tissue caused acutely by ischemia or chronically by neuro-degenerative diseases (Gurney et al., 1996; Levy and Bray, 2003). This may be due to the fact that brain consumes large quantities of oxygen relative to its contribution to total body mass and this with its paucity of oxidative defense mechanisms, place this organ at risk for damage mediated by reactive oxygen species (Skaper et al., 1999). In addition, catecholamine metabolism such as dopamine and norepinephrine and an abundant supply of transition metals make the brain an ideal target for free radical attack (Venarucci et al., 1999). Free radicals can stimulate chain reaction by interacting with proteins, lipids and nucleic acids causing cellular dysfunction and even death due to defective cellular antioxidant defense system (Rukmini et al., 2004).

Protein-energy malnutrition (PEM), a multiple nutrient deficiency syndrome, is a problem, which concerns about half the world's children (Adebayo and Adenuga, 2007). PEM impacts on mental development and cognition in children and also reduces their interaction with environment, which in turn, can lead to problems with attention, inactivity and unresponsiveness. Each of these behaviors compromise children's ability to learn (Ortega et al., 1997). Generally, the antioxidant defense system of protein-undernourished animals and humans are known to be depressed (Adenuga, 2000). The pathogenesis of edema and anemia commonly found in children with PEM has been suggested to be caused by an imbalance between the production of toxic radicals and their safe disposal (Ashour et al., 1999).

It is recognized that enhancing the antioxidant defense system during the rehabilitation of PEM is important to the survival of protein- undernourished subjects. However, while attempts to use some antioxidants such as glutathione have been unsuccessful (Bray and Taylor, 1994), some others for example, selenium have been shown to be effective (Adebayo and Adenuga, 2007). The observed effect of selenium has encouraged us to determine whether zinc alone without protein replenishment could also protect PU subjects from brain damage.

In this study we report that zinc ameliorates the effects of oxidative stress but not absolutely effective in reversing brain damage induced by protein-undernutrition.

MATERIALS AND METHODS
Animals, diets and chemicals

Male weanling albino rats used for the experiment were obtained from the animal house of the University of Agriculture, Abeokuta, Nigeria. Diets were prepared according to Olowookere (1994). Low protein diet contained 5% casein while normal diet contained 16% casein. Casein and all reagents used were of analytical grade and obtained from Sigma Chemical Co., USA and BDH Chemicals Ltd; Poole, England.

Experimental protocol

Animals were randomly assigned to three groups A, B and C. Animals in group A (six rats) were placed on normal (6% casein) diet while the two other groups B and C (of five rats each) were placed on low protein (5% casein) diet. All animals were housed in wire cages. Food and water were given *ad libitum*. At the end of the twelfth week, zinc (zinc acetate) in water (30 mg/l) according to McNall et al. (1995) was given to animals in group C while the other groups (A and B) were given water. Feeding continued for additional two weeks. The animals were sacrificed and brains were dissected out, kept in the freezer at - 4 °C for analyses.

Preparation of crude brain homogenate

A mass of 0.1 g of the brain of individual sample was homogenized in 1ml 0.01M phosphate buffer, pH 7.4, kept frozen in the freezer and used for catalase, glutathione and lipid peroxidation analyses.

Lipid peroxidation assay

Brain lipid peroxidation was carried out by measuring the thiobarbituric acid-reactive (TBAR) products using the procedure of Vashney and Kale (1990).

Glutathione (GSH) Assay

Assay for glutathione (GSH) was done by the method of Beutler et al. (1963).

Catalase assay

The catalase activity of each brain samples was determined by the method of Sinha (1972) but with a slight modification. 0.1 ml of each brain part was mixed with 4.9 ml of distilled water. One milliliter of the mixture was added to hydrogen peroxide–phosphate.

Statistical analysis

The data obtained were analyzed using one-way Analysis of Variance (ANOVA). Differences between means were determined by the use of Duncan multiple range test at 95% confidence level (SPSS Software).

RESULTS

The results of this experiment as presented on Table 1 show that protein undernutrition induced significant reductions (P<0.01) in brain weight, and catalase activity while it induced significant increase (P<0.05) in lipid peroxidation and brain to body weight ratio when compared with well-nourished rats. The ingestion of low protein diet resulted in a reduction in GSH level when compared with well fed rats but it did not reach a statistical significance. However, while the ingestion of zinc acetate in drinking water (30 mg/l) by PU rats for two weeks had no effect on the brain weight, it induced significant reduction (P<0.05) in both lipid peroxidation and GSH level when compared with PU and well fed rats given water only.

The results also showed that brain to body weight ratio of Zn-treated PU rats were not significantly different from PU rats given water only. In addition, there was significant increase (P<0.05) in catalase activity following the ingestion of zinc for two weeks when compared with PU rats. The result of catalase activity in Zn-treated rats were comparable to well fed rats.

DISCUSSION

The effects of malnutrition on brain and brain function have been well established

Table 1: The brain weight and other biochemical parameters of well-fed and protein undernourished (PU) rats.

	Brain wt (g)	Brain wt./Body wt. (g)	Lipid peroxidation (units/g tissue x 10^7)	GSH (mg/g tissue)	Catalase Activity (units/g tissue x 10^5)
Well-fed rats n= 6	1.82 ± 0.14^a	0.0095 ± 0.0014^a	8.00 ± 1.54^a	22.07 ± 5.99^a	9.58 ± 1.80^a
PU rats n= 5	1.48 ± 0.07^b	0.0137 ± 0.0032^b	12.64 ± 1.81^b	15.47 ± 3.75^a	5.10 ± 0.97^b
Zn-treated PU rats n= 5	1.59 ± 0.07^{ab}	0.0151 ± 0.0019^b	4.16 ± 1.74^c	6.67 ± 2.94^b	8.60 ± 2.85^a

Well-fed rats (rats on normal diet) were given diet containing 16% casein and PU rats were given diet containing 5% casein. Zinc (Zinc acetate) was added to drinking water (30 mg/l) after 12 weeks on low protein diet (containing 5% casein) and given *ad libitum* for two weeks. All animals were maintained on their respective diets throughout the experiment.
Values are presented as Means ± SD; values with different superscripts in the same column are statistically different at 95% confidence level.

(Levitsky and Strupp, 1995) and impairments in brain and brain function have been associated with free radicals and antioxidant deficiency in the brain. The result of this experiment showing that the mean brain weight of protein-undernourished (PU) rats is significantly lower than that of well-nourished rats also proved this fact (Adebayo and Adenuga, 2007). The reduction in brain weight has been reported to be associated with a reduction in volume of specific brain areas such as the cerebral cortex and the brain region closely linked to cognitive and intellectual functioning (Levitsky and Strupp, 1995). It has been well established that there is increased lipid peroxidation in PU rats suggesting a role for free radicals in the pathogenesis of protein-undernutrition (Adenuga, 2000). Our present results also support these findings. The observation in this study that Zn supplementation to PU rats for two weeks significantly attenuates lipid peroxidation can be interpreted to mean that zinc protects the brain of PU rats against oxidant or free radical-induced damage and underscores the antioxidant functions of zinc.

Evidence abounds on the fact that free radicals are responsible for the beginning or aggravation of brain diseases (Rayman, 2000; Levy and Bray, 2003). As a matter of fact, various neurodegenerative disorders and syndromes are associated with oxidative stress (Behl and Moosmann, 2002). Our results showing reduced activity of catalase in the brain of PU rats lend credence to this fact. This result is also consistent with various reports on reduced catalase activity in neurodegenerative disease of the brain (Ambani et al., 1975; Aksernov et al., 1998; Batcioglu et al., 2005). However, the brain GSH of PU rats was not significantly affected.

The reduced lipid peroxidation observed in PU rats following zinc acetate supplementation was accompanied with a significant reduction in GSH level and significant increase in catalase activity. The zinc-induced reduction in GSH level suggests the involvement of other antioxidant enzymes induced by zinc and requiring GSH, such as superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) in the scavenging of the free radicals in Zn-treated PU rats which led to the reduction in lipid peroxidation. The increase in catalase activity in Zn-treated PU rats comparable to well fed rats is reflective of the fact that catalase plays a major role in the scavenging of free radicals in zinc-treated PU rats. We had earlier reported that selenium is effective in preventing or ameliorating PU-induced brain damage (Adebayo and Adenuga, 2007). The result of this investigation that zinc supplementation to PU rats for two weeks had no significant ($P > 0.05$) effect on the brain weight is reflective of the fact that zinc is ineffective in reversing PU-induced brain damage. The insignificant difference between the brain to body weight ratio of PU rats and Zn-treated PU rats further buttresses this point.

Conclusively, it appears that zinc is effective in reducing brain lipid peroxidation in rats as a result of increase catalase activity and by a mechanism that requires GSH but ineffective in ameliorating protein-undernutrition-induced reduction in brain weight.

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